SERUM PROTEINS IN DOGS WITH INJURED HEPATIC CIRCULATION

AUTHOR(S):

KURAMOTO, SHINJI

CITATION:

KURAMOTO, SHINJI. SERUM PROTEINS IN DOGS WITH INJURED HEPATIC CIRCULATION. 日本外科宝函 1960, 29(1): 125-156

ISSUE DATE:

1960-01-01

URL:

http://hdl.handle.net/2433/207060

SERUM PROTEINS IN DOGS WITH INJURED HEPATIC CIRCULATION

by

SHINJI KURAMOTO

From the 1st Surgical Division, Kyoto University Medical School
(Director: Prof. Dr. CHISATO ARAKI)
(Received for publication Aug. 31, 1959)

INTRODUCTION

Since ROBERT and others reported on the serum protein fractions in patients suffering from liver diseases, there have been many reports on the close relationship between the serum protein and hepatic functions. Many experimental studies have also been made ··BERRYMANN recognized that partial or total hepatectomy resulted in either a decrease in the serum albumin or impediment to its regeneration; MADDEN pointed out a decrease in albumin in ECK fistula; and MCKEE and others investigated the relationship between the serum protein and the ascites in disturbed hepatic circulation. Electrophoresis has been used by LEUTSCHER and his many followers. For example, MARTIN reported a marked decrease in alpha globulin, not to mention albumin and gamma globulin, in cases of cirrhosis. MUNRO made it clear that no recovery of alpha globulin was observed in hepatectomized animals. As for beta globulin, KUNKEL and many others reported on its increase in many cases of liver diseases.

However, the functions of the liver are so diverse and so complicated that many questions concerning these problems are yet to be answered: What impediment to these functions should result in what changes in the serum proteins? In what mechanism should various changes come? Recent years have seen much progress in the techniques of producing such conditions: ···ECK fistula; the method of constriction of the thoracic inferior vena cava (abbreviated to the thor. i. v. c. hereafter) which was begun by RICHARD and completed by MC KEE; TSUCHIYA's method of constriction of the hepatic veins, a device to obviate the defect, seen in the above mentioned method, of circulation impediment to the areas dominated by the thor. i. v. c., especially to the kidney and adrenal glands; and the method of ligation of the hepatic arteries which MARKOWITZ and others have experimentally made practicable with the use of penicillin.

Using these methods, I have made the following experiment to investigate the serum protein fractions under various conditions of hepatic circulation impediment. I have also investigated the serum protein fractions in dogs injured by repeated doses of CCl_4 which had been widely used as hepatotoxic agent.

II METHOD

1. Operative Procedure

I) Ligation of hepatic arteries

URABE in our clinic has proved that simultaneous section under double ligations of the "3 hepatic aretries", that is, the common hepatic artery, gastroduodenal artery and right gastric artery, interrupts nearly all hepatic arterial supply, with a mortality of almost 100% in dogs. So this method was employed here.

II) Partial constricition of thor. i. v. c.

Some dogs were operated upon with their left sides down under intravenous nembutal anesthesia. For positve pressure respiration, intratracheal intubation was carried out with the tube connected to a simplified artificial respirator. The thorax was then opened between the 5th and 6th right rib to reach the thor. i. v. c., which was pressed slightly with a commercial cellophane band. Penicillin, 100,000 units, was put into the thorax, which was then closed. After natural respiration was recovered, the inserted catheter was taken out. Ascites was observed about a week after the operation. Autopsy finidings disclosed that it was not the cellophane band itself but the granular tissue produced between the band and the venous wall by some heterogenous stimulus that pressed and constricted the vein.

III) Constriction of hepatic veins

Constriction of the thor. i. v. c. causes circulation impediment not only to the liver but also to various organs and tissues situated distal to its constriction, especially such important organs as the kidney and adrenal glands. Therefore TSUCHIYA's method was employed which causes impediment of circulation only to the liver. This method, however, resulted in a high mortality, so it was used with some modifications on it…the middle and left branches of hepatic veins were constricted as the method told us, while the uppermost of the right branches only were subjected to the same procedure, such others as were difficult to be separated from the liver parenchyma being left intact.

IV) Administration of carbon tetrachloride

A half cubic centimeter per kg body weight of 20 to 40% CCl_4 olive oil solution (about 40% solution) was intramuscularly injected every 3 to 8 days (about twice a week) for 12 to 70 weeks.

2. Determination of the Serum Protein and of the Ascitic Protein

Adult mongrel dogs, weighing 7 to 20 kg, were used. Blood samples were taken from the vein in the hind limb. No anti-coagulant was used. The blood was left for about 15 minutes at room temperature, and then centrifuged for 10 minutes at 3,000 rmp to separate serum from the blood. For determination of the total serum protein was used a refractometer made by ERMA, and for fractionation of the serum proteins a TISELIUS electrophoresis apparatus made by "HITACHI". The standard techniques set by "Denki Eidoo Kenkyu Kai in Japan" were follwed:

(1) Serum samples were diluted to between 1.5 g/dl and 2.0 g/dl.

(2) For dilution was used a phosphoric acid buffer solution, a mixture of 1 vol. of 1/10 Mol. KH_2PO_4 and 16 vol. of 1/10 Mol. Na_2HPO_4.

(3) Dialysis was continued for 24 hours in an ice room at 2 to 4°C, with the diluted soluition wrapped in a sheet of commercial cellophane being put in the outer

solution of the above-mentioned buffer.

(4) During the whole process of electrophoresis, the temperature was kept between 4 and 10°C.

(5) The electric current used was 10 mA.

(6) For analysis of electrophoretic patterns, the measurement of area was replaced by that of weight.

Similar procedures were taken in determination of ascitic proteins.

III RESULTS

1. Serum Proteins in Normal Dogs

The serum proteins in normal dogs were determined to serve as the control. It was impossible to have all dogs under the exactly same normal condition, so those dogs which showed no abnormality from the beginning of the observation were considered to be normal, and blood samples were taken comparatively early. The blood of the dog is easy to get hemolytic and those samples which became hemolytic when taken were omitted. No consideration, however, was given to the difference in the season, date or time when the blood samples were taken.

The results of the 15 cases I measured are shown in Table 1. The values of total serum protein lie between 5.0 and 7.6 g/dl, with an average of 6.5 g/dl. These figures are a little lower than that of 7.5 g/dl reported by FUKUDA as the average of 35 cases. As in Table 2 and Fig. 1, fractionation of serum proteins revealed 48.5% for albumin (Al), 16.4% for alpha globulin (α-gl), 20.7% for beta globulin (β-gl) and 14.4% for gamma globulin (γ-gl), all on the average. (The abbreviations to be used in the following pages are given in the brackets.) BOGUTH reported 53.5% for Al, 13.8% for α-gl, 20.4% for β-gl and 12.3% for γ-gl. FUKUDA gave 49.3% for Al, 12.0% for α-gl, 22.3% for β-gl, and 16.4% for γ-gl. There is no marked difference between the results of these three experiments. The A/G ratio was, in most cases, about 1.0, with an average of 0.97.

Table 1 Serum proteins in normal dogs.

Dog No.	11	20	22	25	26	29	37
Total protein	5.8	6.2	7.3	7.2	6.8	7.6	7.4
Albumin g/dl	2.9	3.3	4.2	4.3	3.2	4.3	3.8
Globulin g/dl	2.9	2.9	3.1	2.9	3.6	3.3	3.6
A/G	1.0	1.14	1.36	1.48	0.89	1.30	1.06

39	42	46	56	65	73	74	1 th	Average
6.5	6.5	5.6	5.5	6.5	5.8	7.1	5.0	6.5
3.8	3.0	2.2	2.7	2.6	2.2	3.2	2.1	3.2
2.7	3.5	3.4	2.8	3.9	3.6	3.9	2.9	3.3
1.41	0.86	0.65	0.97	0.67	0.62	0.82	0.72	0.97

Table 2 Fractions of the serum protein in normal dogs.

Dog No.	Albumin		α-Globulin		β-Globulin		γ-Globulin	
	%	g/dl	%	g/dl	%	g/dl	%	g/dl
11	49.3	2.86	19.7	1.14	18.3	0.90	12.7	0.74
20	53.2	03.3	6.2	0.38	21.8	1.35	18.8	1.16
22	56.9	4.16	10.0	0.73	19.7	1.43	13.5	1.98
25	59.2	4.26	1.21	0.87	18.4	1.32	10.3	0.74
26	47.1	3.20	20.0	1.36	20.8	1.41	12.1	0.82
29	56.6	4.30	17.4	1.32	13.0	0.98	13.0	0.98
37	51.0	3.77	19.2	1.42	22.4	1.66	7.4	0.55
39	58.8	3.82	11.8	0.77	17.6	1.14	11.8	0.77
42	46.2	3.00	13.0	0.85	26.0	1.08	14.8	0.97
46	38.6	2.16	18.2	1.02	20.4	1.14	22.8	1.28
56	45.5	2.71	24.2	1.46	22.8	1.38	7.5	0.45
65	40.4	2.62	21.1	1.37	21.9	1.49	16.6	1.08
73	37.8	2.19	21.7	1.25	16.2	1.94	24.3	1.41
74	45.5	3.23	15.9	1.13	25.0	1.78	13.6	0.97
1th	41.2	2.06	16.4	0.82	26.2	1.30	16.4	0.82
Average	48.5	3.17	16.4	1.06	20.7	1.29	14.4	0.92
Boguth	53.5		13.8		20.4		12.3	
Fukuda	49.3		12.0		22.3		16.4	

Fig. 1 Fractions of the serum protein in normal dogs.

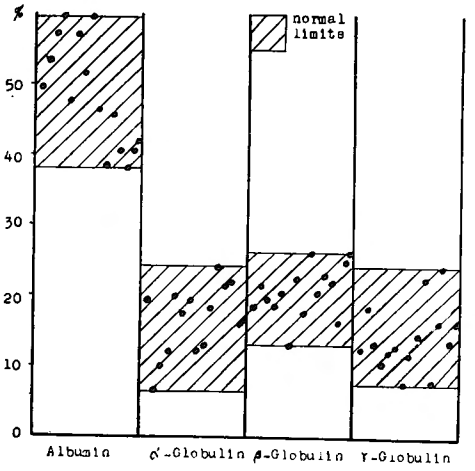
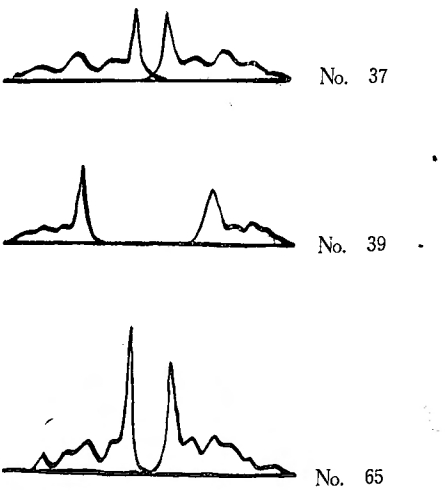


Fig. 2 Electrophoreograms of the serum protein in normal dogs.



It must be mentioned here that dogs differ so much from one another that, as shown in Fig. 1, not the average, but upper and lower limits of all various levels obtained from the control dogs were regarded as normal ones, and these were compared with those obtained from dogs with different hepatic disorders.

2. The Serum Proteins in Dogs with Ligation of the Hepatic Arteries

I) Changes in the serum protein fractions within a short period after ligation

After ligation of the hepatic arteries performed in the above-mentioned way, the serum protein fractions were determined every 6 hours till the 24th hour. As TANTURI reported, almost all dogs, which died of the ligation, revealed hemolysis apparently caused by the poison of rampant anaerobic bacteria.

Those which survived also showed a similar tendency. According to MOORE, electrophoresis after dialysis with a phosphoric acid buffer solution reveals an increase in γ-gl in the fractions of the serum protein with hemoglobin added to it. In the present experiment, however, what I am chiefly concerned with is β-gl, so hemolysis is thought to make no significant difference in the result.

The results of the 9 cases are given in Table 3 and Fig. 3. All showed a remarkable decrease in Al and increase in β-gl as time went on. At the 24th hour following ligation, Case 11 revealed a 22.6% decrease in Al, a 23.9% increase in β-gl, but scarcely any changes in α-gl and γ-gl. In Case 20, 24 hours after ligation, Al decreased 25.7%, α-gl and β-gl increased 13.8% and 18.2% respectively, and γ-gl decreased 8.3%. In Case 72, preoperative electrophoresis of the serum failed, so comparison was made between the values obtained 6 hours and those 24 hours after ligation.

Table 3a Changes in the serum proteins within 24 hours after ligation of the hepatic arteries.

No. 11	Albumin		α-Globulin		β-Globulin		γ-Globulin	
	%	g/dl	%	g/dl	%	g/dl	%	g/dl
Before operation	49.3	3.44	19.7	1.38	18.3	1.28	12.7	0.90
6 hours after operation	40.2	2.82	18.2	1.27	27.3	1.91	14.2	1.00
12 hours after operation	39.2	2.63	17.6	1.18	31.4	2.10	11.8	0.79
18 hours after operation	30.8	2.16	17.3	1.21	42.3	2.96	9.6	0.67
24 hours after operation	26.7	1.87	17.8	1.24	42.2	2.96	13.3	0.93

Table 3 b

No. 20	Albumin		α-Globulin		β-Globulin		γ-Globulin	
	%	g/dl	%	g/dl	%	g/dl	%	g/dl
Before operation	53.2	3.83	6.2	0.45	21.8	1.57	18.8	1.35
6 hours after operation	40.0	2.88	8.0	0.58	36.0	2.59	16.0	1.15
18 hours after operation	34.2	2.46	15.7	1.13	37.6	2.71	12.5	0.90
24 hours after operation	27.5	1.98	20.0	1.44	40.0	2.88	12.5	0.90
16 Days after operation	40.0	2.32	17.5	1.02	39.5	2.36	10.5	0.66

Table 3c

No. 31	Albumin		α-Globulin		β-Globulin		γ-Globulin	
	%	g/dl	%	g/dl	%	g/dl	%	g/dl
Before operation	29.9	1.94	14.3	0.86	42.8	2.76	13.0	0.84
6 hours after operation	31.7	1.90	14.6	0.88	41.5	2.49	12.2	1.73
18 hours after operation	30.0	1.95	16.0	1.04	42.0	2.73	12.0	0.78
24 hours after operation	22.0	1.43	17.0	1.15	47.5	3.08	13.5	0.88
42 hours after operation	24.0	1.56	19.6	1.27	41.2	2.68	15.2	0.98

Table 3d

No. 72	Albumin		α-Globulin		β-Globulin		γ-Globulin	
	%	g/dl	%	g/dl	%	g/dl	%	g/dl
Before operation								
6 hours after operation	48.3	3.14	8.6	0.56	25.9	1.68	17.2	1.12
18 hours after operation	42.5	2.80	13.0	0.86	26.0	1 72	18.5	1.22
24 hours after operation	38.7	2.51	9.7	0.63	32.2	2.10	19.4	1.26
8 days after operation	25.0	1.43	22.9	1.31	35.4	2.01	16.7	0.95

Table 3e

No. 73	Albumin		α-Globulin		β-Globulin		γ-Globulin	
	%	g/dl	%	g/dl	%	g/dl	%	g/dl
Before operation	37.8	2.19	21.7	1.25	16.2	0.94	24.3	1.41
6 hours after operation	35.7	2.14	16.0	0.96	34.0	2.04	14.3	0.86
12 hours after operation	33.3	2.00	18.6	1.11	33.3	2.00	14.8	0.89
18 hours after operation	28.8	2.02	11.2	0.78	45.0	3.15	15.0	1.05
24 hours after operation	28.3	2.04	17.0	1.22	41.5	2.99	13.2	0.95

Table 3 f

No. 74	Albumin		α-Globulin		β-Globulin		γ-Globulin	
	%	g/dl	%	g/dl	%	g/dl	%	g/dl
Before operation	45.5	3.23	15.9	1.13	25.0	1.77	13.6	0.97
6 hours after operation	33.9	2.50	14.5	1.01	37.1	2.58	14.5	1.01
12 hours after operation	28.5	1.95	23.3	1.64	37.5	2.6	10.7	0.76
18 hours after operation	25.0	1.97	19.6	1.55	39.3	2.11	16.1	1.27
9 days after operation	30.7	1.83	19.3	1.15	39.5	2.36	10.5	0.66

Table 3 g

No. 7	Albumin		α-Globulin		β-Globulin		γ-Globulin	
	%	g/dl	%	g/dl	%	g/dl	%	g/dl
Before operation	23.1	1.67	18.5	1.33	46.2	3.32	12.3	0.88
6 hours after operation	30.7	2.00	15.4	1.00	32.8	2.13	21.1	1.37
12 hours after operation	31.4	2.19	11.7	0.84	39.3	2.74	17.6	1.23

Table 3 h

No. 65	Albumin		α-Globulin		β-Globulin		γ-Globulin	
	%	g/dl	%	g/dl	%	g/dl	%	g/dl
Before operation	40.4	2.62	21.1	1.37	21.9	1.43	16.6	1.08
6 hours after operation	32.6	1.86	23.9	1.36	32.6	1.86	10.9	0.62
12 hours after operation	32.1	2.03	26.4	1.66	34.0	2.14	7.5	0.47

Table 3 i

No. 57	Albumin		α-Globulin		β-Globulin		γ-Globulin	
	%	g/dl	%	g/dl	%	g/dl	%	g/dl
Before operation	46.0	2.98	6.2	0.42	39.5	2.56	8.3	0.54
24 hours after operation	27.9	1.81	16.3	1.06	41.9	2.73	13.6	0.90

Table 4a Changes in fractions of the serum protein within 24 hours after ligation of the hepatic arteries. Albumin

Dog No.	Before operation	6 hours after operation	12 hours after operation	18 hours after operation	24 hours after operation
11	49.3%	40.2%	39.2%	30.8%	26.7%
20	53.2	40.0		34.2	27.5
31	29.9	31.7		30.0	22.0
72		48.3		42.5	38.7
73	37.8	35.7	33.3	28.8	28.3
74	45.5	33.9	28.5	25.0	
7	23.1	30.7	31.4		
65	40.4	32.6	32.1		
57		46.0			27.9
Average	39.9	37.7	32.9	31.9	28.5

Table 4b α-Globulin

Dog No.	Before operaiton	6 hours after operation	12 hours after operation	18 hours after operation	24 hours after operation
11	19.7%	18.2%	17.6%	17.3%	17.8%
20	6.2	8.0		15.7	20.0
31	14.3	14.6		16.0	17.0
72		8.6		13.0	9.7
73	21.7	16.0	18.6	11.2	17.0
74	15.9	14.5	23.3	19.6	
7	18.5	15.4	11.7		
65	21.1	23.9	26.4		
57		6.2			16.3
Average	16.8	13.9	19.5	15.5	16.3

Table 4c β-Globulin

Dog No.	Before operation	6 hours after operation	12 hours after operation	18 hours after operation	24 hours after operation
11	18.3%	27.3%	31.4%	42.3%	42.2%
20	21.8	36.0		37.6	40.0
31	42.8	41.5		42.0	47.5
72		25.9		26.0	32.2
73	16.2	34.0	33.3	45.0	41.5
74	25.0	37.1	37.5	39.3	
7	46.2	32.8	39.3		
65	21.9	32.6	34.0		
57		39.5			41.9
Average	27.5	34.1	35.1	38.7	40.9

Table 4 d γ-Globulin

Dog No.	Before operation	6 hours after operation	12 hours after operation	18 hours after operation	24 hours after operation
11	12.7%	14.3%	11.8%	9.6%	13.3%
20	18.8	16.0		12.5	12.5
31	13.0	12.2		12.0	13.5
72		17.2		18.5	19.4
73	24.3	14.3	14.8	15.0	13.2
74	13.6	14.5	10.7	16.1	
7	12.3	21.1	17.6		
65	16.6	10.9	7.5		
57		8.3			13.9
Average	15.9	14.3	12.5	14.0	14.3

The result was: Al decreased 9.6%, β-gl increased 6.3%, but α-gl and γ-gl suffered no changes. In Case 73, 24 hours postoperatively, Al, α-gl and γ-gl showed a decrease of 10.5, 4.7 and 11.1% respectively, while α-gl revealed a 25.3% increase. At the 24th postoperative hour, Case 74 presented a decrease of 20.5% in Al, but an increase of 3.7% in α-gl, 14.3% in β-gl and 2.5% in γ-gl respectively. Case 65 was followed up only till 12 and a half hours after ligation, when it died. At that time Al and γ-gl showed a decrease of 8.3 and 9.1% respectivey, while α-gl and β-gl revealed an increase of 5.3 and 12.1% respectively. In Cases 31 and 7, which had been suffering from a chronic hepatic disorder due to intramuscular injection of CCl_4, a marked decrease in Al and a high increase in β-gl had already been observed before the ligation. Case 7 once showed an increase in Al and a decrease in β-gl 6 hours after ligation, but later again Al declined and β-gl augmented until the 17th postoperative hour, when the animal died, without returning to the preoperative levels. The same was true with Case 31. After having once shown an increment in Al and a decline in β-gl, it showed, on determination after the 12th hour, a decrease in Al and an increase in β-gl.

It was made clear that the first 6 hours saw a rapid, great decrease in Al and a similar increase in β-gl. As for the decrease in Al; at the 6th hour Case 11 showed a 9.1% decrease out of the 22.6% decrease it did in 24 hours; Case 20 a 13.6% decrease out of the 25.7% decrease; and Case 74 a 11.6% decrease out of the 25.5% decrease. As for the increase in β-gl: 6 hours after the ligation Case 11 presented a 14.2% increase out of the 18.2% increase it did in 24 hours; Case 73 a 17.8% increase out of the 25.3% increase; and Case 74 a 12.1% increase out of the 14.5% increase. After the 6th hour it seemed that the Al level dropped and that of β-gl rose as time went on, whereas α-gl behaved erratically···it increased in some cases and decreased in others, but γ-gl revealed little changes.

Tables 4 (a, b, c,) and Fig. 3 show changes in the values of the serum protein fractions and their averages. It is clear that Al decreases and β-gl increases, while no marked changes are seen in either α-gl or γ-gl.

II) The serum proteins in dogs which survived long after the ligation of the

hepatic arteries

Fig. 3 Serum proteins within 24 hours after ligation of the hepatic arteries.

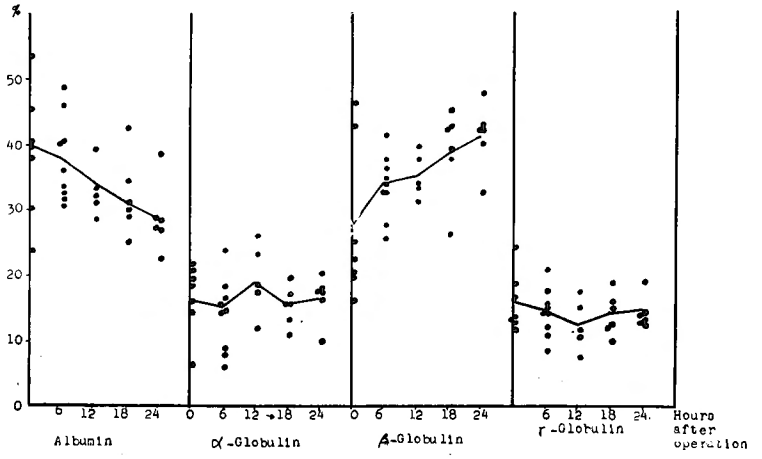


Table 5 Serum proteins in the portal dogs.

Dog No.	Dog after operation	Albumin		α-Globulin		β-Globulin		γ-Globulin		A/G
		%	g/dl	%	g/dl	%	g/dl	%	g/dl	
1	11	36.7	2.31	31.1	1.96	20.0	1.26	12.2	0.77	0.58
2	16	40.6	2.36	15.6	0.90	31.3	1.82	12.5	0.72	0.71
3	180	39.5	2.68	18.4	1.25	28.9	1.97	13.2	0.90	0.65
4	24	35.0	2.27	22.5	1.46	27.5	1.79	15.0	0.98	0.54
5	10	32.3	2.11	20.6	1.34	32.3	2.11	14.8	0.96	0.48
6	19	26.3	1.47	16.2	0.91	38.7	2.17	18.8	1.05	0.36
7	8	25.0	1.43	22.9	1.31	35.4	2.01	16.7	0.95	0.34
8	5	39.1	2.54	15.2	0.99	32.6	2.12	13.1	0.85	0.64
20	16	40.0	2.32	17.5	1.02	27.5	1.59	15.0	0.87	0.67
74	9	30.7	1.83	19.3	1.15	39.5	2.36	10.5	0.66	0.44
Average		34.5	2.13	19.9	1.23	39.5	1.92	14.2	0.87	0.54

Serum proteins were determined on 10 dogs which certainly survived the ligation. The results are shown in Table 5. The Al level lies between 25.0 and 40.6%, with an average of 34.5%; the α-gl level between 15.2 and 31.1%, with an average of 19.9%; the β-gl level between 20.0 and 39.5%, the average being 31.4%; and the γ-gl level between 10.5 and 18.8%, with an average of 14.2%. As in Fig. 5, the decrease in Al and the increase in β-gl are obvious, while no marked changes are seen in the levels of α-gl and γ-gl. In such a comparatively short period as 10 days after the ligation, especially the increase in β-gl was marked···it was over 30% in Cases 5, 7, 8 and 74. On the contrary, in dogs which survived long after the ligation, the β-gl level had returned nearly to the normal. For example, the increase was 27.3% in Case 24 on the 24th day and 28.9% in Case 3 on the 180th day

Fig. 4 Electrophoreograms of the serum protein in portal dogs.

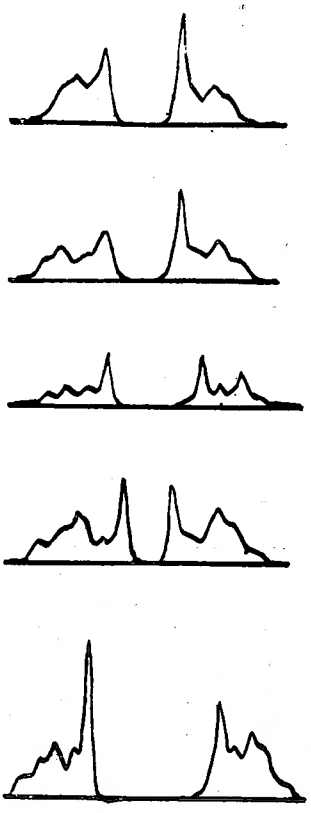
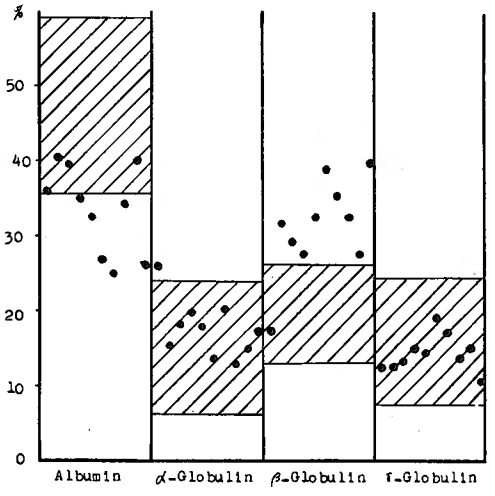


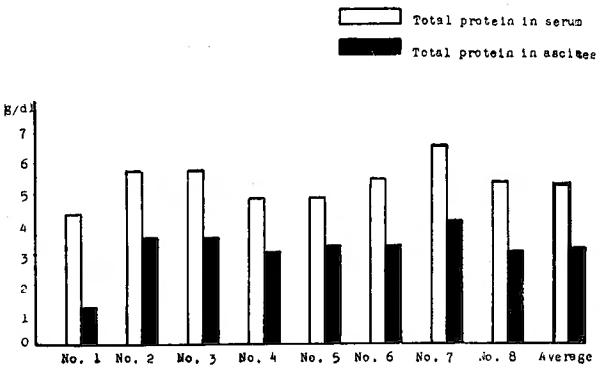
Fig. 5 Fractions of the serum protein in portal dogs.



postoperatively.

The β-gl level increased up to 40.8 % in Case 20, and 32.2% in Case 7, both determined 24 hours after the surgery, while the increase was 39.3% in Case 74 at the 18th hour. Compared with the levels determined on the 16th, 8th and 9th postoperative day, the above figures, showing their highest levels, are believed to have lasted for a considerable long time. The total serum protein displayed a slight tendency to decrease, but with the average of 6.15, the tendency counting for little. The A/G ratio necessarily showed a marked decline, the average being 0.54.

Fig. 6 Serum proteins and ascitic proteins in dogs with constriction of the thoracic inferior vena cava.



3. The Serum and Ascitic Proteins in Dogs with Partial Constriction of the Thor. i. v. c.

I) Serum protein

a) Total serum protein

Fig. 6 shows the total serum protein levels in 8 dogs which formed ascites in about 2 weeks after the constriction previously mentioned. The levels lie between 4.3 g/dl and 6.5 g/dl, with an average of 5.38 g/dl. These figures are lower, compared with the normal mean of 6.5 g/dl. It may be mentioned, by the way, that symptoms were observed suggesting the dilution of blood due to the decline in hematocrit at the time of drawing blood.

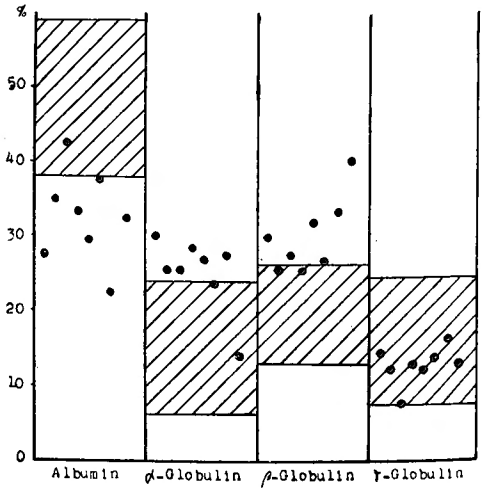
b) Fractions of the serum proteins

As shown in Table 6, Al was 22.2 to 42.6%, with an average of 32.6%; α-gl was 13.5 to 30.0%, with an average of 25.1%; β-gl 25.4 to 40.6%, with an average of 30.1%; and the average of γ-gl was 12.3%. Fig. 7 discloses a decrease in Al, an increase in α-gl and β-gl, but no remarkable changes in γ-gl.

Table 6 Serum proteins in dogs with constriction of the thoracic inferior vena cava.

Dog No.	Albumin		α-Globulin		β-Globulin		γ-Globulin	
	%	g/dl	%	g/dl	%	g/dl	%	g/dl
1	28.0	1.20	30.0	1.29	30.0	1.29	12.0	0.53
2	35.1	2.00	25.7	1.41	25.7	1.41	13.5	0.78
3	42.6	2.43	25.5	1.45	27.7	1.58	4.2	0.24
4	33.3	1.60	28.6	1.37	25.4	1.22	12.7	0.61
5	29.3	1.41	26.8	1.28	31.7	1.52	12.2	0.59
6	37.8	2.02	23.2	1.26	26.8	1.45	14.2	0.77
7	22.2	1.44	27.7	1.80	33.4	2.17	16.7	1.09
8	32.4	1.71	13.5	0.71	40.6	2.15	13.5	0.73
Average	32.6	1.72	25.1	1.32	30.1	1.59	12.3	0.67

Fig. 7 Serum protein fractions in dogs with constriction of the thoracic inferior vena cava.



As shown above, the increase in α-gl and β-gl is marked. Unlike the method of ligation of the hepatic arteries or that of constriction of the hepatic veins, the method of constriction of the thor. i. v. c. causes congestion and other lesions to important organs other than the liver, such as the kidney or adrenal glands. Therefore, the changes in the serum proteins brought about by the constriction were partly similar to those seen with renal ascites in case of neprosis etc. Thus it is assumed that

the increase in α-gl was more related with the kidney and others than with the liver.

a) Total ascitic protein

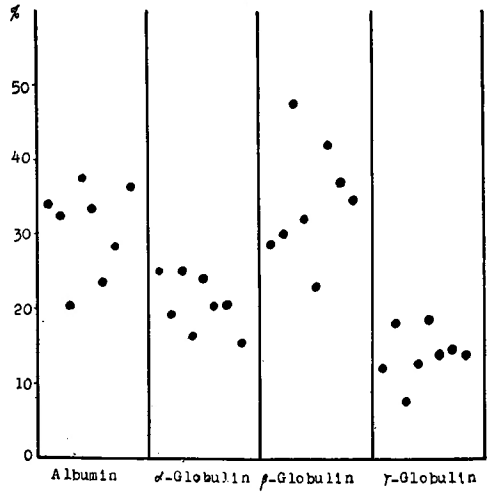
The total ascitic protein ranges from 1.2 to 4.0 g/dl, with an average of 3.1 g/dl, exceeding the highest level as a transudate (Fig. 6).

b) Fractions of the ascitic proteins

Table 7 Proteins in ascites of dogs with constriction of the thoracic inferior vena cava.

Dog No.	Albumin		α-Globulin		β-Globulin		γ-Globulin	
	%	g/dl	%	g/dl	%	g/dl	%	g/dl
1	34.6	0.41	25.0	0.30	28.8	0.35	11.6	0.14
2	33.0	1.15	19.0	0.67	30.0	1.05	18.0	0.63
3	20.6	0.72	25.0	0.87	47.3	1.66	7.1	0.25
4	37.5	1.12	15.9	0.48	34.1	1.03	12.5	0.37
5	33.7	1.08	24.4	0.78	23.3	0.75	18.6	0.59
6	23.6	0.75	20.4	0.65	42.0	1.34	14.0	0.44
7	28.6	1.14	20.6	0.84	36.5	1.46	14.3	0.56
8	36.1	1.08	15.3	0.46	34.7	1.04	13.9	0.42
Average	29.7	0.93	20.7	0.63	34.6	1.08	13.7	0.43

Fig. 8 Proteins in ascites of dogs with constriction of the thoracic inferior vena cava.



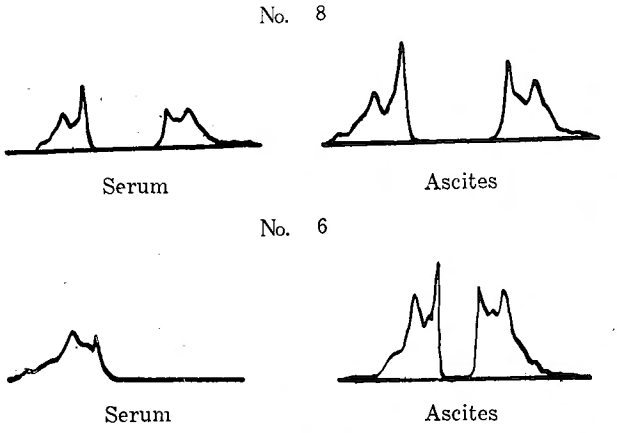
As in Table 7 and Fig. 8, the Al fraction ranges from 20.6 to 37.5%, with the mean of 29.7%: the α-gl fraction from 15.3 to 25.0%, with the mean of 20.7%; the β-gl from 23.3 to 47.3%, with the mean of 34.6%; and the γ-gl fraction from 7.1 to 18.6%, with the mean of 13.7%. A comparison of these figures with those of ascitic dogs has been made in Fig. 9, showing a similar tendency between the two. From this it is clear that the serum and the ascites are closely related with each other.

It may be especially noteworthy that the proportion of β-gl to the other fractions in the ascitic proteins is larger than that in the serum proteins, while that of α-gl is a little smaller in the former than in the latter.

III) The serum proteins in the ascitic dogs with an added ligation and section of the hepatic arteries

First, constriction of the thor. i. v. c. was performed. About 2 weeks latter 5 dogs which were found to have developed about 1.5 to 3.0 liter ascites, were further subjected to ligation and section of the hepatic arteries. From them blood samples

Fig. 9 Electrophoreograms of the serum protein and the ascitic protein in ascitic dogs.



were taken at intervals of 6 hours for determination of the serum proteins. After the ligation 300,000 units of penicillin were daily injected into Cases 1 and 3, which were able to survive 90 and 75 hours respectively···longer than otherwise. Cases 4, 5 and 6 were not treated with penicillin and died at the 12th, 12th and 14th hour respectively. The autopsy, however, disclosed no necrotic changes grossly, except only congestion in the liver. The levels of the fractions of the serum proteins are given in Table 8 (a, b, c, d, e) and Fig. 10 (a, b). In Case 1, Al showed little changes throughout the course of observation: it was 28.0% before ligation, and after it, it turned out to be 27.1% at the 12 th hour, 29.2% at the 18th hour and 29.6% at the 24th hour. In Case 3 the level had a slight tendency to decrease as time went on: that is, it began with 42.6% before ligation, and postoperatively, after showing 38.5% at the 6th hour and 37.2% at the 12th hour, it finally came down to 32.0% at the 24th hour. Case 4 showed no great changes in the levels··· 33.3% preoperatively, and 33.4% at the 6th hour and 33.4% at the 12th hour (1 hour before the death) after ligation. Case 5 revealed 29.3% before ligation and after surgery, 39.6% at the 6th hour and 29.2% at the 12th hour (just before the death). And in Case 6, the level showed a slight inclination toward decline···37.8%

Table 8a Serum proteins after ligation the hepatic arteries in ascitic dogs.

No. 1	Albumin		α-Globulin		β-Globulin		γ-Globulin	
	%	g/dl	%	g/dl	%	g/dl	%	g/dl
Before operation	28.0	1.20	30.0	1.29	30.0	1.29	12.0	0.53
6 hours after operation								
12 hours after operation	27.1	1.17	18.6	0.80	40.7	1.75	13.6	0.58
18 hours after operation	29.2	1.11	27.1	1.03	31.2	1.18	12.5	0.48
24 hours after operation	29.6	1.12	31.8	1.21	25.0	0.95	13.6	0.52

Table 8b

No. 3	Albumin		α-Globulin		β-Globulin		γ-Globulin	
	%	g/dl	%	g/dl	%	g/dl	%	g/dl
Before operation	42.6	2.43	25.5	1.45	27.7	1.58	4.2	0.24
6 hours after operation	38.5	2.16	24.6	1.38	32.3	1.81	4.6	0.25
12 hours after operation	37.2	1.86	23.6	1.18	29.4	1.47	9.8	0.49
18 hours after operation								
24 hours after operation	32.0	1.76	24.0	1.32	30.0	1.65	14.0	0.77

Table 8c Serum protein after ligation of the hepatic arteries in ascitic dogs.

No. 4	Albumin		α-Globulin		β-Globulin		γ-Globulin	
	%	g/dl	%	g/dl	%	g/dl	%	g/dl
Before operation	33.3	1.60	28.6	1.37	26.1	1.25	12.0	0.58
6 hours after operation	33.4	1.60	27.2	1.31	29.0	1.39	10.4	0.50
12 hours after operation	33.4	1.60	23.0	1.10	33.5	1.61	10.1	0.49

Table 8d

No. 5	Albumin		α-Globulin		β-Globulin		γ-Globulin	
	%	g/dl	%	g/dl	%	g/dl	%	g/dl
Before operation	29.3	1.41	26.8	1.28	31.7	1.52	12.2	0.59
6 hours after operation	39.6	1.90	24.5	1.17	22.7	1.08	13.2	0.65
12 hours after operation	29.2	1.28	20.8	0.92	37.5	1.65	12.5	0.55

Table 8e

No. 6	Albumin		α-Globulin		β-Globulin		γ-Globulin	
	%	g/dl	%	g/dl	%	g/dl	%	g/dl
Before operation	37.8	2.02	23.2	1.26	26.8	1.45	14.2	0.77
6 hours after operation	27.5	1.47	20.4	0.83	44.8	1.79	6.9	0.28
12 hours after operation	21.4	0.86	20.0	0.80	44.3	1.77	14.3	0.57

Fig. 10a Serum proteins after ligation of the hepatic arteries in ascitic dogs

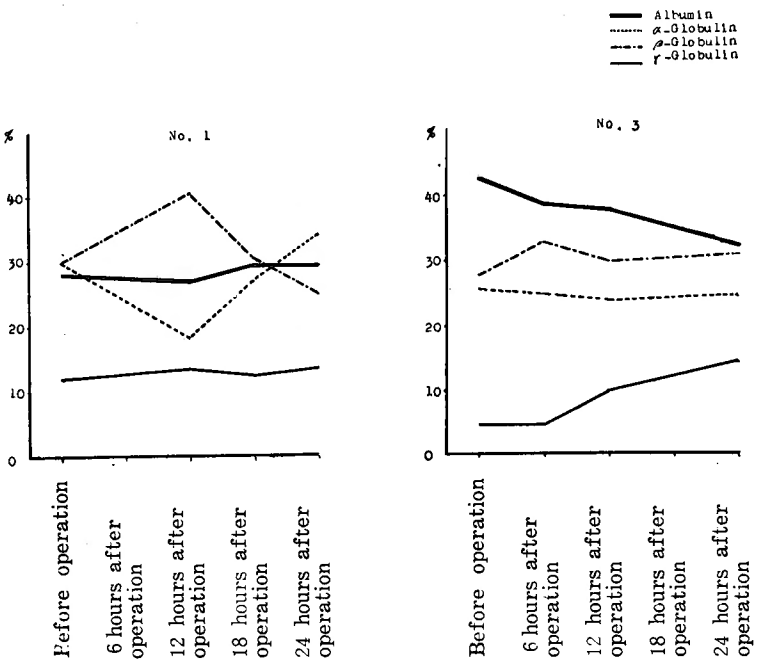
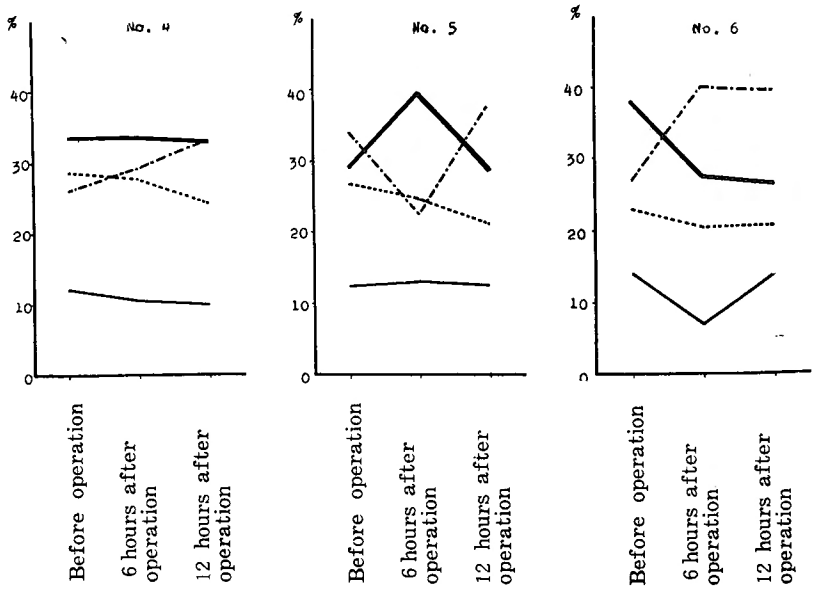


Fig. 10b Serum proteins after ligation of the hepacic arteries in ascitic dogs.



before surgery, and after it, 27.5% at the 6th hour and 21.4% at the 12th hour (2 hours before the death).

As for α-gl and β-gl, electrophoretic separation (of these fractions from the others) became more difficult as the death approached. α-gl behaved erratically··· in Case 1, it showed 30.0% before ligation, and after it, 18.6% at the 12th hour, 27.1% at the 18th hour and 31.8% at the 14th hour. In Case 3, however, it disclosed no marked changes···25.5% preoperatively, and 24.6% at the 6th, 23.6% at the 12th, and 24.0% at the 24th postoperative hour. In Case 4, it began with 28.6 % before ligation, and postoperatively slightly decreased from 27.2% at the 6th hour to 19.0% at the 12th hour. Case 5 showed a decrease from preoperative 26.8% to 24.5% at the 6th and 20.8% at the 12th postoperative hour. Case 6 revealed a slight tendency to decrease ···23.2% before ligation, and 20.6% at the 6th hour, 20.0% at the 12th hour postoperatively.

As for β-gl; in Case 1, it once showed an increase but later a tendency to decrease; that is, the preoperative level was 30.0%, and after ligation, it went up to 40.7% at the 6th hour, but after passing a level of 31.2% at the 12th hour, it came down to 25.0% at the 24th hour. In Case 3, however, no remarkable changes were seen; before ligation the level was 27.7%, and postoperatively it was 32.3% at the 6th, 29.4% at the 12th and 30.0% at the 24th hour. In Case 4, the trend was for the level to rise as time went on···31.6% before ligation, 29.0% at the 6th and 36.5% at the 12th postoperative hour. Case 5 showed 31.7% before surgery, and postoperatively dropped to 22.7% at the 6th hour, but came back with 37.5% at the 12th hour···a level higher than the level reached before ligation. Case 6 revealed 26.8% preoperatively, and after ligation, went quite high up to 44.8% at the 6th and 44.3% at the 12th hour.

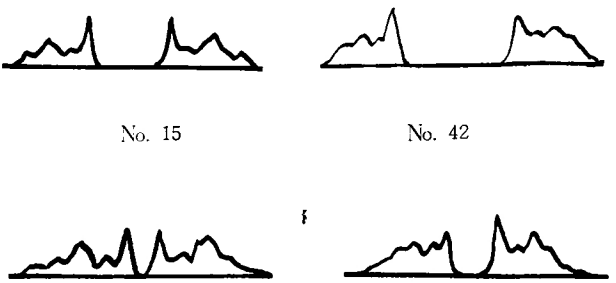
In summary, in penicillin treated dogs, Cases 1 and 3, the β-gl level showed no striking changes, although to temporary increase was observed. On the other hand, in untreated Cases 4, 5 and 6, the level once dropped, but later showed a tendency to rise gradually until their death.

4. The Serum Proteins in Dogs with Constriction of the Hepatic Veins

It is known that Mc KEE dogs (with constriction of the thor. i. v. c.) begin to form ascites about a week after the operation, which increases to reach the maximum amount in about 2 weeks. So, in the present experiment, determination of the serum proteins were performed on the dogs which had survived over 2 weeks after the constriction. By the way, in any of them ascites was not recognized before the death.

The total serum protein levels showed scarcely any changes, lying between 5.7 g/dl and 7.3 g/dl, with the mean level of 6.6 g/dl. Table 9 and Fig. 12 show the levels of the serum protein fractions. Al shows a decrease, with 22.8% to 39.2% and an average of 32.7%; α-gl suffers no changes, with 13.5% to 25.3% and an average of 17.9%; β-gl reveals a marked increase, with 25.5% to 40.0% and an average of 32.9%; and γ-gl shows no changes, with 10.7% to 22.8% and an average of 16.5%. Case 55 presented a peculiar electrophoretic pattern, with 25.3% for α-gl and 22.8%

Fig. 11 Electrophoreograms of the serum protein in dogs with constriction of the hepatic veins.



No. 15 No. 42

No. 40 No. 48

Table 9 Fractions of the serum protein in dogs with constriction of the hepatc veins (2weeks postoperatively)

Dog No.	Albumin		α-Globulin		β-Globulin		γ-Globulin	
	%	g/dl	%	g/dl	%	g/dl	%	g/dl
15	37.9	2.46	13.5	0.88	37.9	2.46	10.7	0.70
40	29.0	1.65	14.6	0.83	40.0	2.27	16.4	0.94
42	39.2	2.56	19.6	1.27	25.5	1.65	15.7	1.02
48	34.7	2.46	15.9	1.13	31.9	2.27	17.4	1.24
55	22.8	1.67	25.3	1.85	29.1	2.12	22.8	1.61
Average	32.7	2.15	17.9	1.19	32.9	2.15	16.5	1.11

for γ-gl.

5. The Serum Proteins in Dogs with Hepatic Lesions Caused by a Long Term Injection of CCl_4

The method of injection of CCl_4 was described above. Determinations of the serum proteins were performed on dogs with chronic hepatic lesions caused by the injection. For convenience of investigation, results obtained were summed up every 5 weeks throughout the course of injection (every 10 injection).

I) The serum proteins in dogs within 5 weeks after the commencement of intramuscular CCl_4 injection (less than 10 injection)

Table 10 shows the total serum protein levels in 8 dogs which received less than 10 injections of CCl_4 intramuscularly. They range from 4.8 g/dl to 7.3 g/dl, with an average of 6.2 g/dl, which are not very different from the normal average of 6.5 g/dl. However, the amount of Al is 2.5 g/dl···a lower level than the normal 3.2 g/dl. This decrease in Al, in its turn, brings about a slight relative increase in total globulin the amount of which is 3.7 g/dl. Therefore, a reduction of the A/G ratio results.

The levels of the serum protein fractions are given in Table 11 and Fig. 13. Al shows a slight decrease, with 28.6 to 46.0% and an average of 37.1%; α-gl

Table 10 Serum proteins in dogs within 5 weeks of the intramuscular injection of CCl_4.

Dog No.	15	17	22	23	27	33	34	35	Average
Total protein	5.8	6.2	7.3	6.2	4.8	7.1	6.1	6.3	6.2
Albumin g/dl	2.7	1.8	3.9	1.8	2.1	2.4	1.8	2.7	2.5
Globulin g/dl	3.1	4.4	3.4	4.4	2.7	4.7	4.3	3.6	3.7
A/G	0.83	0.40	0.73	0.42	0.86	0.50	0.42	0.72	0.61

Table 11 Serum protein fractions in dogs within 5 weeks of the intramusculer injection of CCl_4.

Dog No.	Albumin		α-Globulin		β-Globulin		γ-Globulin	
	%	g/dl	%	g/dl	%	g/dl	%	g/dl
15	46.0	2.70	10.0	0.58	25.5	1.48	20.0	1.16
17	28.6	1.77	17.9	1.10	39.5	2.45	14.3	0.89
22	42.3	3.90	23.0	1.68	23.0	1.68	11.5	0.83
23	29.6	1.83	22.2	1.47	37.0	2.27	11.2	0.68
27	45.9	2.11	18.8	0.90	18.8	0.90	15.2	0.71
33	33.3	2.36	19.4	1.38	22.2	1.58	25.2	1.77
34	29.2	1.78	29.2	1.78	25.0	1.53	16.6	1.01
35	42.0	2.65	18.4	1.16	18.4	1.16	21.2	1.30
Average	37.1	2.50	19.8	1.22	26.2	1.54	16.9	0.94

Fig. 12 Serum proteins in dogs with constriction of the hepatic veins.

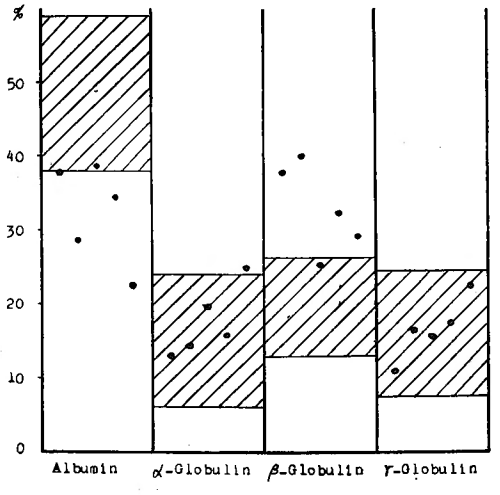
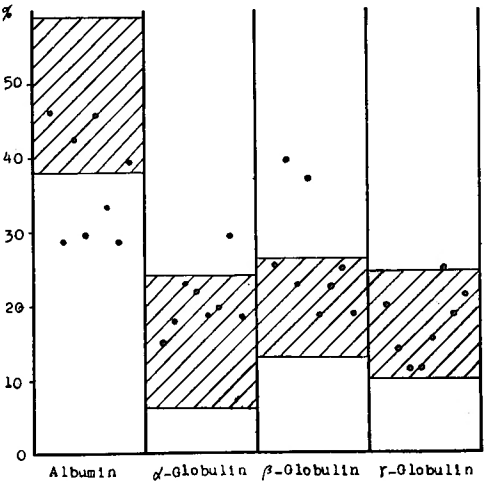


Fig. 13 Serum proteins in dogs within 5 weeks after the commencement of intramuscular CCl_4 injection



reveals no changes, with 10.0% to 29.2% and an average of 19.7%; β-gl discloses a slight increase, with 18.0 to 39.5% and an average of 26.2%; and γ-gl suffers no changes, with 11.2% to 25.0% and an average of 16.9%.

II) The serum proteins in dogs from 5 to 10 weeks after the commencement of intramuscular CCl_4 injection (10 to 20 injection)

Table 12 presents the levels determined on the 5 dogs which received 10 to 20

intramuscular injections of CCl_4. The total serum protein levels show no changes, ranging from 5.9 to 7.5 g/dl, with an average of 6.7 g/dl. On the other hand, the Al level drops, with 4.3 g/dl; and the total Gl level shows an increment, with 4.3 g/dl; and the A/G ratio is at quite a reduced level of 0.59.

The levels of the serum protein fractions are shown in Table 13 and Fig. 14. Al reveals a slight decrease, with 35.0 to 40.0% and an average of 36.5%; α-gl suffers no changes with 15.0 to 23.2% and an average of 18.0%; β-gl discloses a slight

Table 12 Serum proteins in dogs from 5 to 10 weeks of the intramuscu larinjection of CCl_4.

Dog No.	2	3	26	31	33	Average
Total protein	6.7	5.9	7.3	7.5	6.0	6.7
Albumin g/dl	2.4	2.1	2.7	3.0	2.4	2.4
Globulin g/dl	4.3	3.8	4.6	4.5	3.6	4.3
A/G	0.56	0.55	0.57	0.67	0.70	0.59

Table 13 Serum protein fractions in dogs from 5 to 10 weeks of the intramuscular injection of CCl_4.

Dog No.	Albumin		α-Globulin		β-Globulin		γ-Globulin	
	%	g/dl	%	g/dl	%	g/dl	%	g/dl
2	35.2	2.36	17.5	1.17	30.2	2.03	17.1	1.17
3	35.0	2.07	17.2	1.01	34.0	2.01	13.8	0.81
26	36.4	2.66	18.2	1.32	30.3	2.21	15.1	1.10
31	40.0	3.00	15.0	1.13	30.0	2.25	15.0	1.12
33	39.3	2.36	23.2	1.39	17.8	0.77	19.7	1.18
Average	36.5	2.43	18.0	1.19	29.1	1.93	16.5	1.14

Fig. 14 Serum proteins in dogs from 5 to 10 weeks after the commencement of the intramuscular CCl_4 injection.

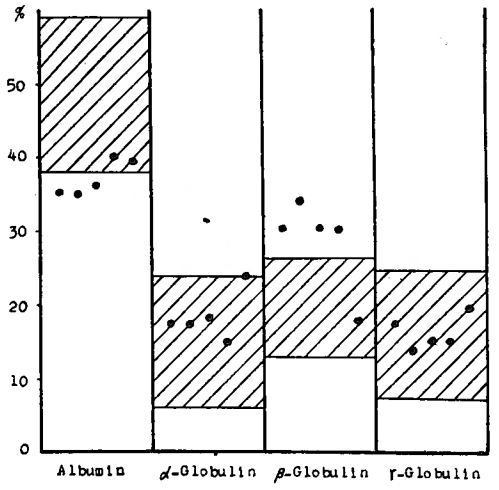


Fig. 15 Serum proteins in dogs from 10 to 15 weeks of the intramuscular injection of CCl_4.

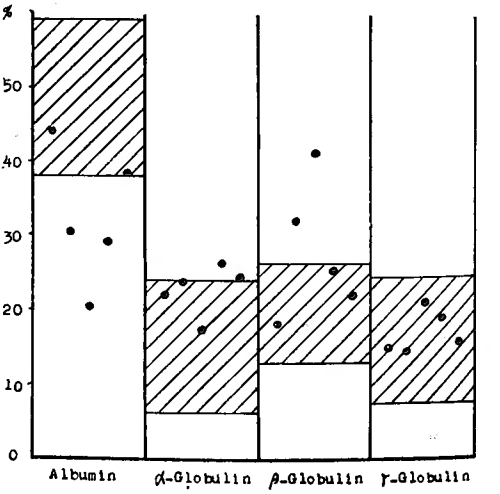


Table 14 Serum proteins in dogs from 10 to 15 weeks of the intramuscular injection of CCl_4.

Dog No.	10	14	18	24	32	Average
Total protein g/dl	8.2	6.9	5.8	6.9	5.8	6.7
Albumin g/dl	3.2	2.1	1.2	1.9	2.2	2.2
-Globulin g/dl	4.6	4.8	4.6	5.0	3.6	4.5
A/G	0.79	0.43	0.26	0.40	0.62	0.50

Table 15 Serum protein fractions in dogs from 10 to 15 weeks of the intramuscular injection of CCl_4.

Dog No.	Albumin		α-Globumin		β-Globumin		γ-Globulin	
	%	g/dl	%	g/dl	%	g/dl	%	g/dl
10	44.2	3.62	22.2	1.82	18.2	1.52	15.0	1.23
14	30.2	2.10	23.2	1.62	32.2	2.22	14.3	0.99
18	20.7	1.20	17.2	0.99	41.4	2.40	20.7	1.20
24	28.7	1.93	26.6	1.84	25.5	1.76	19.2	1.32
32	38.5	2.23	24.1	1.40	21.7	1.26	15.7	0.91
Average	32.5	2.26	22.6	1.53	27.9	1.83	17.0	1.13

Table 16 Serum priotens in dogs from 15 to 20 weeks of the intramuscular injection of CCl_4.

Dog No.	2	4	11	20	22	Average
Total protein g/dl	5.7	7.0	7.7	6.2	7.7	6.86
Albumin g/dl	1.18	1.11	2.25	2.63	2.81	2.00
Total globulin g/dl	4.52	5.89	5.45	3.57	4.89	4.86
A/G	0.26	0.19	0.41	0.87	0.62	0.47

increase, with 17.8 to 34.0% and average of 29.1%; and γ-gl shows no changes with 13.8 to 19.7% and an average of 16.5%.

III) The serum proteins in dogs from 10 to 15 weeks after the commencement of the intramuscular CCl_4 injection

The results obtained from 5 cases are given in Table 14. The total serum protein levels show no changes, lying between 5.8 and 8.2 g/dl, with the mean level of 6.7 g/dl. The mean levels of Al and total Gl are 2.2 g/dl and 4.5 g/dl respectively, bringing the A/G ratio down to 0.50.

Table 15 and Fig. 15 present the levels of the serum protein fractions. Al is reduced to a slight degree, with 20.7 to 44.2% and an average of 32.5%; α-gl is slightly elevated, with 17.2 to 26.6% and an average of 22.6%; β-gl increases, with 18.6 to 41.4% and an average of 27.9%; and γ-gl shows no striking changes, with 14.3 to 20.7% and an average of 17.0%.

IV) The serum proteins in dogs from 15 to 20 weeks of the intramuscular injection of CCl_4

In 5 cases, the total serum protein levels range from 5.7 to 7.7 g/dl, with 6.9 g/dl on the average, showing no changes, while Al is reduced to 2.0 g/dl, and Gl is remarkably elevated to 4.86 g/dl, the A/G ratio being reduced very much to 0.47 (Table 16).

As for the serum protein fractions; Al shows a marked decrease, with 15.9 to 41.7% and an average of 28.8%; α-gl suffers no changes, with 10.3 to 19.7% and an average of 15.4%; β-gl is highly elevated, with 27.2 to 58.0% and an average of 40.9%; and γ-gl reveals no changes, with 8.7 to 19.8% and an average of 14.9% (Table 17, Fig. 16).

V) The serum proteins in dogs receiving intramuscular injection of CCl_4 for 20 to 30 weeks

Six cases were traced. The total serum protein levels showed no changes, lying between 4.6 and 7.5 g/dl, with an average of 6.65 g/dl; and the A/G ratio stood as low as 0.49 (Table 18).

As for the serum protein fractions; Al showed a decrease, ranging from 21.7

Table 17 Serum protein fractions in dogs from 15 to 20 weeks of the intramuscular injection of CCl_4.

Dog No.	Albumin		α-Globulin		β-Globulin		γ-Globulin	
	%	g/dl	%	g/dl	%	g/dl	%	g/dl
2	20.7	1.18	10.3	0.59	49.2	2.80	19.8	1.13
4	15.9	1.11	17.4	1.22	58.0	4.06	8.7	0.60
11	29.2	2.25	19.7	1.52	33.6	2.59	17.5	1.35
20	41.7	2.62	16.6	1.05	27.5	1.74	14.1	0.89
22	36.5	2.81	12.7	0.98	36.5	2.81	14.3	1.10
Average	28.8	2.00	15.4	1.07	40.9	2.79	14.9	1.01

Fig. 16 Serum proteins in dogs from 15 to 20 weeks of the intramuscular injection of CCl_4.

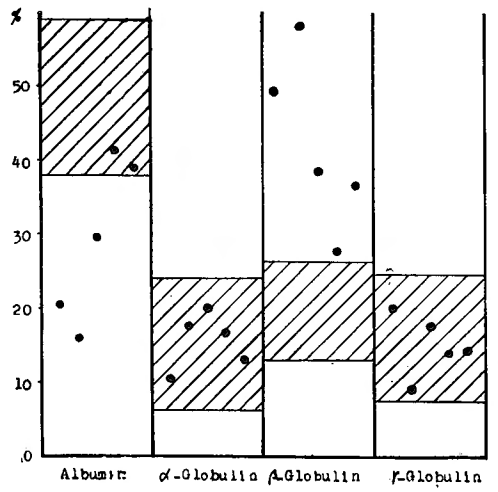


Fig. 17 Serum proteins in dogs from 20 to 30 weels of the intramuscular injection of CCl_4.

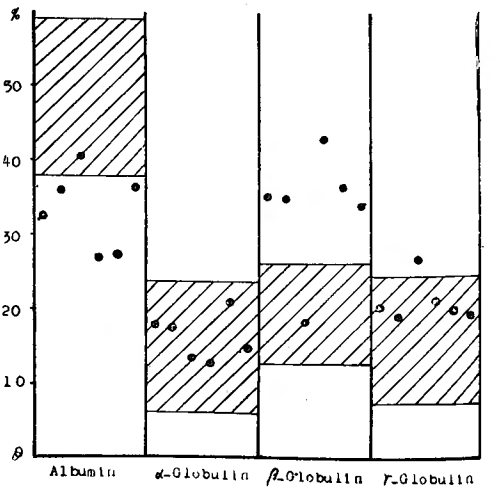


Table 18 Serum proteins in dogs from 20 to 30 weeks of the intramuscular injection of CCl_4.

Dog No.	4	6	7	12	14	15	Average
Total protein g/dl	7.0	6.8	7.2	7.5	4.6	6.8	6.65
Albumin g/dl	2.3	2.4	2.9	2.0	1.3	2.5	2.23
Total protein g/dl	0.47	0.55	0.69	0.30	0.38	0.57	0.49
A/G	4.7	4.4	4.3	5.5	3.3	4.3	4.42

Table 19 Serum protein fractions in dogs from 20 to 30 of the intramuscular injection of CCl_4.

Dog No.	Albumin		α-Globulin		β-Globulin		γ-Globulin	
	%	g/dl	%	g/dl	%	g/dl	%	g/dl
4	32.8	2.3	18.9	1.5	32.8	2.3	15.5	1.1
6	35.7	2.4	17.8	1.2	32.2	2.2	14.3	1.0
7	40.9	2.9	13.6	1.0	18.2	1.3	27.3	2.0
12	27.1	2.0	12.7	1.0	43.5	3.3	16.7	1.3
14	27.4	1.3	21.2	1.0	36.4	1.7	15.0	0.6
15	36.4	2.5	14.8	1.0	34.0	2.3	14.8	1.0
Average	33.4	2.2	16.5	1.1	32.8	2.2	17.3	1.2

Table 20 Serum proteins in dogs receiving the intramuscular injection of CCl_4 for over 30 weeks.

Dog No.	2	4	6	7	9	11	Average
Total protein	7.3	7.5	7.3	7.3	6.5	7.0	7.10
Albumin g/dl	1.82	2.18	2.64	3.18	3.25	2.95	2.67
Globulin g/dl	5.48	5.32	4.66	4.12	3.25	40.5	4.48
A/G	0.37	0.41	0.56	0.94	1.00	0.76	0.67

to 40.9%, with an average of 33.4%; α-gl lay between 12.7 and 21.2%, with an average of 16.5%; β-gl revealed an increase, ranging from 18.2 to 43.5%, with an average of 32.8%; and γ-gl stood between 14.3 and 27.3% with an average of 17.3% (Table 19, Fig. 17).

VI) The serum proteins in dogs receiving intramuscular injections of CCl_4 for over 30 weeks

Six cases belong to this group. The total serum protein levels lie between 6.5 and 7.5 g/dl, with an average of 7.15 g/dl···a little higher than the normal average of 6.5 g/dl. The Al level is 2.67 g/dl and the total globulin level 4.48 g/dl, which results the slight reduction of the A/G ratio to 0.67 (Table 20).

As for the serum protein fractions; Al lies between 25.0 and 50.0%, with an average of 33.7%; α-gl between 11.9 and 21.3%, with an average of 16.3%; β-gl between 22.7 and 45.8%, with 32.1% on the average; and γ-gl between 12.5 and 15.7%, with an average of 13.9%. A marked decrease in Al but a striking increase in β-gl are seen (Table 21, Fig. 18).

As shown in Fig. 20, throughout the whole course of investigation, the tendency was that the more injections of CCl_4 were given, the lower Al levels and the higher

Table 21 Serum protein fractions in dogs receiving the intramuscular injection of CCl_4 for over 30 weeks.

Dog No.	Albumin		α-Globulin		β-Globulin		γ-Globulin	
	%	g/dl	%	g/dl	%	g/dl	%	g/dl
2	25.0	1.82	18.8	1.37	40.5	2.95	15.7	1.15
4	29.2	2.18	12.5	0.97	45.8	3.44	12.5	0.97
6	36.1	2.64	21.3	1.56	29.8	2.18	12.8	0.93
7	43.5	3.18	19.7	1.44	23.7	1.73	13.1	0.96
9	50.0	32.5	11.9	0.77	22.7	1.48	15.4	1.00
11	42.2	2.95	13.7	0.96	30.4	2.13	13.7	0.96
Average	37.7	2.67	16.3	1.18	32.1	2.32	13.9	0.99

β-gl levels resulted. No significant changes were found in α-gl and γ-gl. An increase in β-gl was particularly predominant when about 40 injections were performed, after which the level dropped in spite of additional injections of CCl_4. This may be partly because those dogs which had been able to survive long term hepatic disorders caused by a lot of CCl_4 were especially sturdy…this may also be inferred from the fact that about that time the total serum proteins were on the increase in those dogs…and partly because autumn came around and provided the animals with a favourable environment.

Fig. 18 Serum proteins in dogs receiving the intramuscular injections of CCl_4 for over 30 weeks.

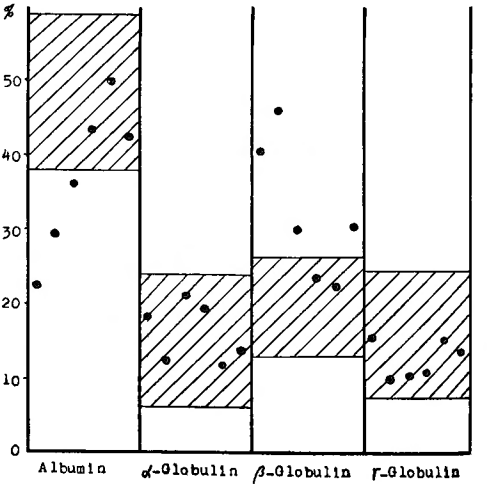


Fig. 19 Electrophorecograms of the serum protein in dogs with hepatic lesion caused by a long term injection of CCl_4. (40% CCl_4 olive oil solution) (0.5cc/kg. 2 /lw)

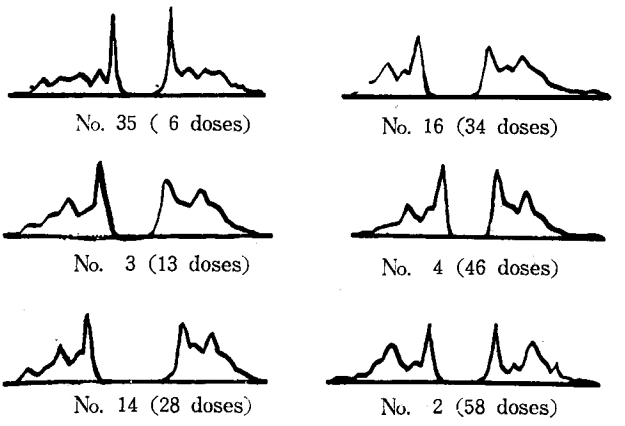
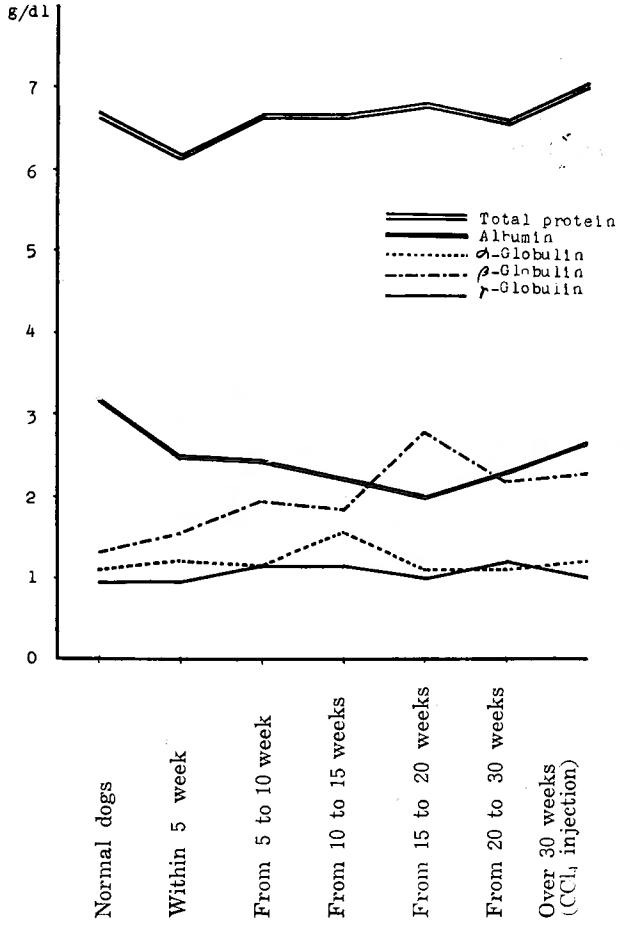


Fig. 20 Changes of the serum proteins in dogs with hepatic lesions caused by a long term injection of CCl_4.



IV DISCUSSION

On the basis of HERRICK's experiment on hepatic irrigation and the fundamental studies made by MARKOWITZ, RAPPAPORT and SCOTTS, and by DAVIS, TANTURI and TARKINGTON, for the first time RIENHOFF clinically took up ligation of the hepatic arteries as a treatment of cirrhosis with ascites. On the other hand, various experimental investigations have been made on the method of the ligation by LAUFMAN, TANTURI, BARAT and others. It has been noticed that when ligation of the hepatic arteries are performed on normal dogs, even antibiotic therapy cannot reduce the mortality below 30%. MARKOWITX, TANTURI and others attribute their death to the α-toxin caused by rampant anaerobic bacteria, while LAUFMAN and others to the absence of collateral arterial circulation, but they all suggest some other factors to be involved in the cause of death.

On the other hand, according to URABE, in our clinic, 100,000 units of penicillin

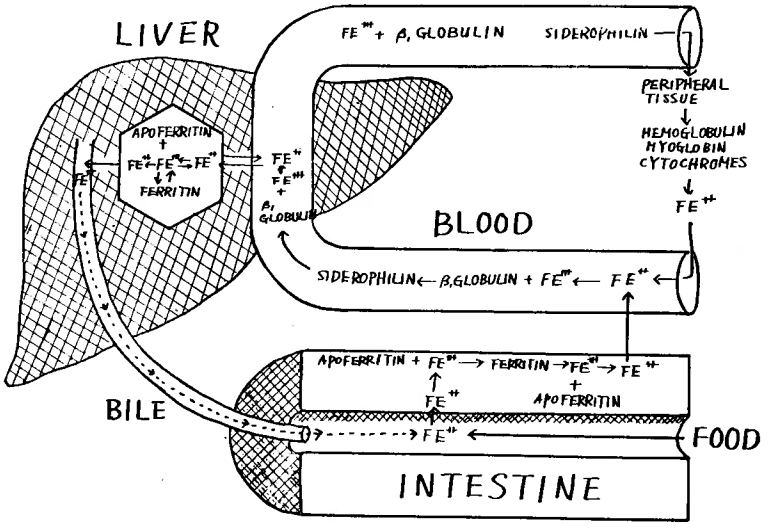
are necessary and sufficient to be given after the ligation of the hepatic arteries. He has also reported the gross observation that immediately after the ligation the liver changes its color to dark purplish red all over its surface; that as time passes, there comes a tendency that the areas which encounter the circulation impediment get localized, and that necrosis begins to develop in these localized areas of congestion. It may be assumed that this temporary circulation impediment plays an important role in the development of liver necrosis as a forerunner of the growth of anaerobic bacteria.

Attaching importance to this point, NAKASE, in our clinic, made experiments on VDM, that is, hepatic ferritin, as a factor involved in circulation impediment. In parallel with this, I made the present study to investigate changes in the serum proteins at the time of hepatic circulation impediment.

It is generally known that the liver plays an important role in the metabolism and storage of iron in the body. Fig. 21 shows the relationship between the liver and iron, quoted from "Liver" by POPPER and SCHAFFNER, Iron is stored in the liver as ferritin, that, when released into the blood, bacomes a form combined with β-gl.

Among chief lesions caused by the ligation of hepatic arteries are oxygen deficiency due to lack of arterial blood and the stasis of portal circulation. Under these conditions, as previously stated, Al decrease and β-gl increases as time passes during the observation of 24 hours. Now, as for the relationship between ferritin and β-gl, NAKASE in our clinic, KOSHIZUKA and others made studies, which tell us that the ligation of the hepatic arteries causes ferritin stored in the liver to be released into the blood stream. It has also been made clear by HEILMEYER's method of measuring ferritin by means of electrophoresis that in the serum protein fractions β-gl increases

Fig. 21



Relation of the liver to the pathways of iron metabolism
(From original of Fig. 20. Popper & Schaffner. LIVER)

as ferritin is released into the blood stream and serum iron increases. Further, OKAMURA, HEILMEYER and others studied and reported on the combination of serum iron with β-gl, using radioactive iron. According to them, it may be assumed that the increase in β-gl following the ligation of the hepatic arteries has close relationship with the release of ferritin into the blood stream.

The increase observed in the β-gl fraction in those dogs which survived long after the ligation of the hepatic arteries may prove the continuance of hepatic circulation impediment. However, after more than 10 days following ligation the β-gl level shows a tendency to return to normal as days pass. This means that towards this time the circulation impediment gradually begins to recover.

In ascitic dogs which had been subjected to McKEE's method of constriction of the thor. i. v. c., all areas in distal to the constriction suffered from congestion.

Especially the kidney and adrenal glands, which play an important role in water metabolism, were seriously much affected by the constriction. Therefore, the changes in the serum proteins had not been caused exclusively by the hepatic circulation impediment. However, the hepatic circulation impediment following the constriction of the thor. i. v. c. is similar to that following the ligation of the hepatic arteries in that both operations cause congestion to the liver. NARITOMI made a report on the serum proteins in dogs under such conditions. The results he obtained are much the same as those I obtained ··· a decrease in Al and an increase in both α-gl and β-gl. The increase in β-gl may be assumed to have been caused by the release of hepatic ferritin into the blood stream due to hepatic circulation impediment. On the other hand, the increase in α-gl may be considered to have been brought about by some changes in the kidney, adrenal glands and other viscera rather than in the liver. This, as well as similar phenomena seen with nephrosis etc., needs further investigation.

These ascitic dogs were further subjected to the ligation of the hepatic arteries. In 2 cases of them which received penicillin, β-gl showed much the same level as before the arterial ligation, and they survived longer than otherwise···until the 75th to 90th postoperative hour. On the contrary, 3 cases without penicillin died all within 14 hours, with a higher β-gl level than before the ligation. The ascitic dogs had already had some ferritin in the blood before the ligation, but after it, it may be assumed, the penicillin to some extent prevented more ferritin from coming out of the liver, so that the worsening of the hepatic circulation inpediment was checked. In the penicillin untreated dogs, it is thought that after the ligation, as well as before it, ferritin continued to be released into the blood until the advancement of the hepatic lesions caused them to die.

The ascitic proteins have some correlation with the serum proteins, with the only difference in β-gl between the two··· in the former the level is higher than in the latter.

BOLTON and BOLWIER reported an increase in hepatic lymph and formation of ascites from the liver capsule at the time of constriction of the thor. i. v. c. FREEMANN, MALLET-GUY and others reported that if the thor. i. v. c. was constricted

after transposing the liver into the thorax through the diaphragm, no fluid collected in the peritoneal cavity, but some only in the thorax. From these reports it may be inferred that an elevation of the intrahepatic portal pressure causes increased hepatic lymph to transude through the liver capsule to form ascites.

McKee made an experiment on absorption of the ascites, in which he injected albumin and globulin, both labeled with C^{14}, into the peritoneal cavity of ascitic dogs. The result was that the Al passed through the peritoneum at least 3 times faster than globulin. This may be proof that Al with smaller molecular weight is easy to be absorbed, while β-gl and other globulins with higher molecular weights are apt to remain in the cavity. Furthermore, according to Davis, the serum protein fractions obtained from the human corpse within 48 hours after death are able to be separated, just as are those taken from the living body. Moreover, he noticed a marked increase in the β-gl level in the serum of the corpse. The conditions under which the blood of the corpse finds itself, such as oxygen deficiency and stasis of blood circulation, are similar to the conditions under which ascites is seen, and it is intersting to note that in both an increment in β-gl is observed.

McKee's method of constriction has a defect that it causes circulation impediment to viscera other than the liver. Honjo and Tsuchiya in our clinic improved on this point and devised a mehtod of constriction of the hepatic veins, which can produce ascitic dogs to the present purpose. In the present experiment, 5 dogs with the hepatic veins constricted by means of our own modification of the method clearly revealed an increase in the β-gl fraction. This proves that they had hepatic lesions, although no ascites was seen.

Following is the summary of the above description. Either ligation of the hepatic arteries, or constriction of the thor. i. v. c. or of the hepatic veins brings about hepatic disorders, which elevate the β-gl level in the serum protein fractions. This elevation is assumed to have been caused chiefly by the release of hepatic ferritin into the blood stream. This fact should be considered with close relationship to, on the one hand, Matassarin, Peterson and others' reports on an increase in the serum iron in some cases of acute hepatitis, and to, on the other, Gray and Baron, Staub and other's reports that the β-gl fraction of the serum proteins often increases in hepatitis. And Aoyagi's report that there is some correlation between the serum iron and the β-gl fraction can be explained by the fact that hepatic ferritin is released in case of hepatic disorders.

In McKee dogs receiving penicillin, no increase in β-gl was observed after the additional ligation of the hepatic arteries, and the hours of survival were prolonged. This may be because sudden and rapid development of the circulation impediment could be prevented by the antibiotics. And according to Baez, Mazur and Shorr, hepatic ferritin already decreases in cirrhosis, so that the ligation of the hepatic arteries causes little, if any, more of it to be released from the liver. This may support the ligation of the hepatic arteries as apossible treatment of cirrhosis. In fact, a human case of cirrhosis experienced in our clinic revealed a constant low β-gl level, showing no changes before and after ligation of the hepatic arteries.

In dogs with chronic hepatic disorders caused by carbon tetrachloride, Al drops while β-gl rises. According to HIMSWORTH, injection of CCl_4 causes lesions only in the peripheral zone of liver lobules; hepatic cells became so swollen and edematous as to press and constrict arterial capillary vessels in the lobules, the central zones of which, deprived of arterial blood supply, become necrotic. This mechanism, as he asserts, is similar to that accroding to which ligation of the hepatic arteries causes central necrosis to the liver through oxygen deficiency. WAKIM reported that injection of CCl_4 caused reflective contraction to occur in intrahepatic capillary vessels. By microscopic observation on the living tissue, NATA found that CCl_4 produced swollen and edematous cells in the intermediate zone of liver lobules, which obstructed the blood flow towards the central zone. In addition, in his report on a case of acute CCl_4 poisoning, HEILMEYER showed a sudden increase in the serum iron···over 1,700 r/dl.

From these studies it is obvious that hepatic disorders caused by CCl_4 are similar to those brought about by the ligation of the hepatic arteries, and that the release of hepatic ferritin naturally follows to cause an increase in β-gl.

V. CONCLUSION

In the present experiment, hepatic disorders were brought about to dogs by the ligation of the hepatic arteries, or constriction of the thoracic inferior vena cava or the hepatic veins, in order to investigate the serum and ascitic proteins. The serum proteins in dogs with chronic hepatic disorders caused by long term injection of CCl_4 were also examined. The results are as follows:

1) In 15 normal dogs, the serum protein levels lie between 5.0 and 7.6 g/dl, with an average of 6.5 g/dl. As for the serum protein fractions: Al is 48.5%, α-gl 16.4%, β-gl 20.7% and γ-gl 14.4%, all on the average.

2) In 9 dogs with the ligation of the hepatic arteries, the Al fraction dropped but the β-gl rose, both to a high degree, as time passed within the short postoperativeperiod of 24 hours. In the 10 cases which survived as long as from 5 days to 6 months, at first Al showed a decrease and β-gl an increase, but as days went on they both disclosed a tendency to return to normal.

3) In 8 ascitic dogs with the constriction of the thor. i. v. c., the total serum protein levels revealed a decrease, with an average of 5.2 g/dl. As for the serum protein fractions: Al showed a decrease, while α-gl and β-gl an increase. The mean level of the total ascitic protein was 3.1 g/dl, higher than the highest level as a transudate. The fractions of the ascitic proteins disclosed much the same tendency as those of the serum proteins, that is, Al was low and α-gl and β-gl high, and especially the increase in β-gl was marked.

4) In 5 cases which survived constriction of the hepatic veins over 2 weeks, the Al fraction decreased, while the β-gl fraction elevated.

5) In dogs with hepatic disorders caused by a long term injection of CCl_4, the determination of the serum protein fractions revealed a decrease in Al and an increase in β-gl.

6) Increases were observed in the β-gl fraction in dogs with hepatic circulation impediment, especially congestion of portal flow, caused by the various methods abovementioned. It may correctly be said that hepatic ferritin released from the liver into the blood stream plays an important role in the increases in the β-gl fraction.

I wish to express my deep gratitude to Prof. Dr. CHISATO ARAKI and Prof. Dr. ICHIO HONJO for their guidance throughout this experimental study. I am also greatly indebted to Assist. Prof. Dr. SYUNSAKU OOSHIMA for his guidance in method of electrophoresis.

References

1) Aoyagi, T.: Studies on Iron Metabolism of Injured Liver. The Japanese Journal of Gastroenterology, **54**, 687, 1957.
2) Aoyagi, T.: Studies on the Relationship of Serum Iron to Serum Copper and Serum Protein Fraction in Liver Diseases. The Japanese Journal of Gastroenterology, **56**, 287, 1959.
3) Baret, A. C. and Fitts, W. T.: Ligation of the Hepatic Artery in Experimental Cirrhosis of the Liver with Portal Hypertension. Surg. Gyn. & Obst., **98**, 33, 1955.
4) Berrymann, G. H., Bollmann, J. L. and Mann, F. C.: The Influence of the Liver on the Protein of the Blood Plasma. Am. J. Physiol., **139**, 556, 1943.
5) Beaz, S., Mazur, A. and Shorr, E.: Hepatorenal Factors in Circulatory Homeostasis. XX: Antidiuretic Action of Hepatic Vasodepressor VDM (Ferritin). Am. J. Physiol., **162**, 198, 1950.
6) Davis, D. R.: Paper Electrophoretic Studies of Postmortem Serum Proteins. Am. J. Med. Sciences, **236**, 472, 1958.
7) Davis, L., Tanturi, C. A. and Tarkington, J.: The Effect of Reduced Blood Flow to the Liver in Renal Hypertension. Surg. Gyn. & Obst., **89**, 360, 949.
8) Erikson, C. C. et al.: Blood Plasma Proteins as influenced by Liver Injury induced by Carbon Tetrachloride and Gum Acacia. Am. J. Path., **14**, 537, 1938.
9) Freeman, S.: Recent Progress in Physiology and Biochemistry of the Liver. M. Clin. North America, **37**, 109, 1953.
10) Heilmyer, L.: Die klinische Bedeutung der Serumkupfer und Serumeisenbestimmung und Ferritinstudien. Saishin Igaku (in Japan), **12**, 110, 1955.
11) Huggins, C. and Post, J.: Experimental Subtotal Ligation of Arteries supplying the Liver. Arch. Surg., **35**, 878, 1937.
12) Haberer, H.: Experimentelle Unterbindung der Leberarterie. Arch, Klin, Chir., **78**, 557, 1905.
13) Himsworth, H. P.: The Liver and its Disease, 2nd Ed. Cambridge. Massachusetts Havard University Press, 1950.
14) Herrick, F. C.: An Experimental Studies into the Cause of Increased Portal Pressure in Portal Cirrhosis. J. Exp. M., **9**, 93, 1907.
15) Fukuda, J.. Electrophoretic Study of Serum Proteins in Dog. Journal of the Japan Veterinary Medical Association, **8**, 66, 1955.
16) Kunkel, H. G. and Slater, R. J.: Lipoprotein Patterns of Serum obtained by Zone Electrophoresis. J. Clin. Jnvest., **31**, 677, 1952.
17) Koshizuka, H.: Studies on Iron Metabolism, especially on Ferritin in the Liver. J. J. S. S. (Nihongekagakkai Zasshi in Japan), **56**, 368, 1955.
18) Laufman, H. et al.: Graded Hepatic Arterial Ligations in Experimental Ascites. Surg. Gyn. & Obst., **96**, 409, 1953.
19) Madden, J. L.: Ligation of the Hepatic and Splenic Arteries in the Treatment of Cirrhosis of the Liver. Surg. Gyn. & Obst., **96**, 594, 1953.
20) Mallet-Guy, P., Devic, G., Feit, R., Soler, A. and Blondent, P.: Experimental Study of Ascites caused by Posthepatic Venous Stenosis; Ligature of the Subhepatic veins. Lyon. Chir., **47**, 807, 1952.
21) Martin, N. H.: The Interrelations of the Serum Proteins in the Liver Damage, with Special Reference to the Thymol Test. J. Clin. Path., **2**, 275, 1949.

22) Markowitz, J., Rappaport, A. M. and Scott, A. C.: The Function of the Hepatic Artery in the Dog. Am. J. Digest. Dis., Oct., 1949.
23) Markowitz, J., Rappaport, A. M. and Scott, A. C.: Prevention of the Liver Necrosis following Ligation of Hepatic Artery. Proc. Soc Exp. Biol., **70**, 305, 1949.
24) Matassarin, B. M. and Delp, M. H.: The Reletion of Serum Iron to Hepatocellular Damage. Am. J. M. Sc., **622**, 221, 1952.
25) McKee, F. W., Schilling, J. A., Tishhoff, G. H. and Hyatt, R. E.: Experimental Ascites, Effects of Sodium Chloride and Protein Intake on Protein Metabolism of Dogs with Constricted Inferior Vena Cava. Surg. Gyn. & Obst., **89**, 529, 1949.
26) McKee, F. W., Yuile, C. L., Lamson, B. G. and Wipple, G. H.: Albumin and Globulin Circulation in Experimental Ascites, Relative Rates of Interchange between Plasma and Ascitic Fluid Studies with C^{14} Labelled Proteins. J. Exp. Med., **95**, 161, 1952.
27) Moore, D. H., Roberts, J. B. et al.: Factors influencing the Electrophoretic Analysis of Human Serum. J. Biol. Chem., **180**, 1147, 1949.
28) Nata, K.: Biomicroscopic Studies on the Relation between the Attitude of the Capillaries and the Location of Injury in Liver in Mice with Chemical Toxins. The Japanese Journal of Gastroenterology, **55**, 1, 1958.
29) Naritome, Y.: Studies on Experimental Ascites Centering around Hepatic Lymph. The Japanese Journal of Gastroenterology, **54**, 625, 1957.
30) Peterson, R. E.: Plasma Radioactive Iron Turnover in Acute Viral Hepatitis. Proc. Soc. Exper. Biol. & Med., **84**, 47, 1953.
31) Peterson, R. E. and Ettinger, R. H.: Radioactive Iron Absorption in Siderosis. Am. J. Med., **15**, 518, 1953.
32) Popper, H., Bean, W. B., de la Huerga, J., Franklin, M., Tsumagari, Y., Routh, J. I. and Steigmann, F.: Electrophoretic Serum Protein in Hepatobiliary Disease. Gastroenterology, **17**, 138, 1951.
33) Popper, H. and Schaffner, F.: Liver; Structure and Function. Mc Graw-Hill Book company, Inc. New York, 1957.
34) Rienhoff, W. F.: Ligation of the Hepatic and Splenic Arteries in the Treatment of Portal Hypertension; with a Report of 6 Cases. Bull. Johns Hopkins Hosp., **88**, 368, 1951.
35) Rienhoff., W. F. and Woods.: Ligation of the Hepatic and Splenic Arteries in Treatment of Cirrhosis with Ascites. J. Am. M. Ass., **152**, 687, 1953.
36) Roberts, S. and white, A.: Studies on the Origin of the Serum Protein. J. Biol. Chem., **180**, 505, 1949.
37) Schilling, J. A., Mc Coord, A. B., Clausen, S. W., Troup, S. B. and McKee, F. W.: Experimental Ascites; Studies of Electrolyte Balance in Dogs with Partial and Complete Occulsion of the Portal Vein and of the Vena Cava above and below the Liver. J. Clin. Invest., **31**, 702, 1952.
38) Shorr, E., Baez, S., Zweifach, B. W., Payne, M. A. and Mazur, A.: The Antidiuretic Actions of the Hepatic Vasodepressor Ferritin (VDM) and its Occurence in Conditions associated with Antidiuresis in Man. Tr. A. Am. Physicians, **63**, 39, 1950.
39) Tanturi, C., Swigart, L. L. and Canepa, J. F.: Preventation of Death from Experimental Ligation of the Liver (Hepatic Proper) Branches of the Hepatic Artery. Surg. Gyn. & Obst., **91**, 680, 1950.
40) Traver, H. and Reinhardt, W. O.: Methionine Labeled with Radioactive Sulfur as an Indicator of Protein Formation in the Hepatectomized Dog. J. Biol. Chem., **167**, 395, 1947.
41) Tsuchiya, R.: Experimental Study of Cirrhosis of the Liver Arch. Jap. Chir., **28**, 1147, 1959.
42) Urabe, H.: The Interruption of the Arterial Flow to the Liver; an Experimental Study. Arch. Jap. Chir., **28**, 1112, 1959.
43) Volwier, W., Grindley, J. H. and Bollmann, J. L.: The Relation of Portal Vein Pressure to the Formation of Ascites; an Expermental Study. Gastroenterology, **14**, 40, 1950.

和文抄録

肝循環障害犬の血清蛋白

京都大学医学部外科学教室第1講座（指導：荒木千里教授）

倉本信二

血清蛋白が肝機能と密接な関係のある事は1925年頃より肝疾患患者の血清蛋白に関する数々の報告がある．実験的にも肝切除或は肝剔出時，Eck 氏瘻形成時，胸腔内下大静脈狭窄時等に際しての血清蛋白の変化を追究している．然しながら肝機能は複雑多岐にわたつていて何れの障害により血清蛋白に如何なる変化が生ずるか，或は如何なる機序によつて各種の変化が現われるかは明確にされていない事項が多い．

胸腔内下大静脈狭窄法，肝静脈狭窄法更に肝動脈結紮法等の実験方法の進歩により，肝循環障害の諸様相を創り出せる様になつた．

著者は此等の方法によつて，各種肝循環障害時の血清蛋白（特に Tiselius 装置による血清蛋白分屑）の検索を行つた．

更に実験的肝障害の目的に屢々使用される四塩化炭素による慢性肝障害時の血清蛋白分屑に就いても再検討を加えた．

実験方法並びに実験結果

1） 正常犬15例の血清総蛋白は 5.0～7.6g/dl で平均 6.5g/dl となつた．血清蛋白分屑は Albumin（以下 Al と略す）48.5%，α-globulin（以下 α-gl と略す）16.4%，β-gl 20.7%，γ-g 14.4%の平均値を示した．従つて A/G は1.0前後のものが多く．平均値 0.97 となつた．

2） 肝動脈遮断は総肝動脈，胃十二指腸動脈，右胃動脈の所謂三肝動脈結紮切断を行つた．遮断後6時間毎に24時間迄の血清蛋白分屑の検索を行つた．9例の測定結果を平均値で示すと，術前値 Al 39.9%，α-gl 16.8%，β-gl 27.5%，γ-gl 15.9% に対し，術後24時間値はAl 28.5%，α-gl 16.3%，β-gl 40.9%，γ-gl 14.3%となり，時間の経過と共に Al の減少，β-gl の増加が著明であつた．5日～半年の長期確実生存例10例の血清蛋白分屑に於ても平均値は Al 34.5%，α-gl 19.9%，β-gl 31.4%，γ-gl 14.2%となり，Alの減少，β-gl の増加を示した．しかし約2週間以上の長期になるに従つて正平値近く迄恢復の傾向を示した．

3） McKee 変法によつて胸腔内下大静脈狭窄を行つた8例の腹水犬の血清総蛋白量の平均は 5.2g/dl で減少を示した．血清蛋白分屑は平均値 Al 32.6%，α-gl 25.1%，β-gl 30.1%，γ-gl 12.3% となり，Al の減少，α-gl 及び β-gl の増加を示した．腹水蛋白量は平均 3.1g/dl で濾出液としての最高濃度以上の値を示した．腹水蛋白分屑は血清のそれと略同一傾向を示し，Al 29.7%，α-gl 20.7%，β-gl 34.6%，γ-gl 13.7%，の平均値で Al は少く，α-gl，β-gl の割合が大で，特に β-gl の増加が著しい．

4） 土屋氏法により肝静脈狭窄を行い，2週間以上生存した犬（腹水は貯溜せず）5例の測定平均値は Al 32.7%，α-gl 17.9%，β-gl 32.9%，γ-gl 16.5% で Al の減少，β-gl の増加を示した．

5） 四塩化炭素投与法としては 40% CCl_4 オリーブ油溶液を体重1kg当り 0.5cc宛，週2回で12週～70週に亘つて筋注した．測定結果は血清総蛋白量は全観察期間を通じて著変は認められなかつたが，血清蛋白分屑は注射回数の増加と共に Al の減少，β-gl の増加の傾向を示した．

肝動脈結紮後，門脈循環障害が起り，肝フェリチンが血中に移行する事，又体内に於ける鉄は肝にフェリチンの形で貯蔵され，血中に移行した場合 β-gl 分屑と結合している事は諸家の研究報告がある．即ち著者の種々の方法による実験的肝循環障害犬の血清蛋白に就いて共通的な事は Al の減少，β-gl の増加であり，この β-gl の増加は肝内門脈循環障害のための肝フェリチンの血中移行が主役割を示していると考えられる．

又四塩化炭素中毒によつて肝フェリチンの血中移行のための血清鉄の著増に関して Heilmyer の報告がある．四塩化炭素中毒によつて肝小葉中心帯が乏血状態に陥り，肝動脈結紮時の低酸素血症による中心帯壊死の発生機序と同様に解されている事より，この際血清蛋白分屑に於ける β-gl の増加も Fernitin の血中移行のためと考えられる．

以上を総括的に述べると，肝動脈結紮，胸腔内下大静脈狭窄，肝静脈狭窄及び四塩化炭素中毒による肝循環障害犬の血清蛋白分屑に於ては Al の減少，β-gl の増加が認められ，この β-gl の増加は肝フェリチンの血中移行が主役割をなしていると思考される．